Elemental bio-imaging: *In situ* analysis of trace elements in tissue by laser ablation – inductively coupled plasma – mass spectrometry

by

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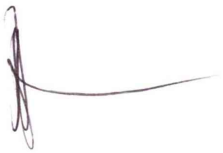
Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.



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This thesis is dedicated to my godmother Mary McFall, with the hope that it may add to the global effort aiming to help treat the millions suffering from Parkinson's disease, just like her.

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D. Hare, F. Burger, C. Austin, F. Fryer, R. Grimm, B. Reedy, R. Scolyer, J Thompson, P. Doble. *Elemental bio-imaging of melanoma in lymph node biopsies.* The Analyst, DOI:10.1039/B812745J

D. Hare, B. Reedy, F. Fryer, R. Grimm, J. George, S. Wilkins, D. Finkelstein, P. Doble. *Elemental bio-imaging: in situ analysis of trace elements in tissue sections.* Invited oral presentation at the Asia-Pacific Winter Plasma Conference on Plasma Spectrochemistry, Tsukuba, Japan, November 2008.

D. Hare, B. Reedy, M. Dawson, F. Fryer, R. Svenningsson, R. Grimm, P.E. Andren, P. Doble. *Metal-imaging mass spectrometry (MIMS): A new imaging mass spectrometry technology to determine the distribution of metal ions in tissue samples.* Oral presentation at the Joint 4th Asia Oceania Human Proteome Organization and 2nd Pacific Rim International Congress on Protein Science, Cairns, Australia, June 2008.

D. Hare, C. Austin, D. Bishop, A. Beavis, B. Reedy, M. Dawson, F. Fryer, R. Svenningsson, X. Zhang, R. Grimm, P. Andren, P. Doble. *Metal Imaging Mass Spectrometry: laser ablation-inductively coupled plasma-mass spectrometry imaging of 6-ohda induced Parkinson's disease and melanoma in lymphatic tissue*. Awarded 2nd place poster prize at the Royal Australian Chemical Institute Research and Development Conference, Adelaide, December 2007.

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D. Hare, B. Reedy, M. Dawson, F. Fryer, R. Grimm, P. Andren, P. Doble. *Trace element imaging of 6-OHDA induced Parkinson's disease in rat brains using laser ablation ICP-MS.* Poster presentation at the European Winter Plasma Conference on Plasma Spectrochemistry, Taormina, Sicily, Italy, March 2007.

D. Hare, P. Doble. *Metal Imaging Mass Spectrometry: In situ analysis of trace elements in neurological tissue using laser ablation ICP-MS*. Oral presentation at the Australian and New Zealand Society for Mass Spectrometry Conference, Christchurch, New Zealand, January 2007.

Abstract

Elemental bio-imaging is a new application of laser ablation – inductively coupled plasma – mass spectrometry (LA-ICP-MS) that determines *in situ* trace element concentrations in thin sections of biological tissues. This project developed a LA-ICP-MS method for creating colour images of the regional distribution of both metals and non-metals in a variety of biological samples.

The developed method was capable of producing images with a lateral resolution as low as 30 μm. This was achieved using a 30 μm laser spot that was rastered across the sample at a rate of 30 μm s^{-1}. It was found that a laser fluence of 0.22-0.28 J cm^{-2} was best suited for soft tissue sections with minimal particle redeposition and fracturing of the surrounding sample. Evaluation of the octopole reaction system (ORS) fitted to the Agilent 7500 ce instrument found that the use of a collision gas for interference removal was inadvisable for imaging experiments. Experiments were carried out to determine the significance of potential polyatomic interferences in the ablation of tissue. It was found that the 'dry' nature of the plasma in LA-ICP-MS significantly reduced the occurrence of O and H based polyatomics, and the small sample load in each ablation reduced the effect of other matrix-based interferences on elements in the mg kg^{-1} concentration range.

Application of the method to sections of human lymph nodes impregnated with malignant melanoma found imaging of ^{31}P was able to accurately discern healthy cells from cancerous tissue. Measurement of the ratio between ^{31}P and other elements improved the contrast between the two types of cells. Three-dimensional models of imaged lymph nodes further improved the distinction between the melanoma cells and normal tissue.

A method was developed for producing matrix-matched tissue standards by homogenising chicken tissue with known amounts of added elemental standards. Digestion of the tissue standards was performed and analysis by solution nebulisation ICP-MS confirmed the concentration of each added element. The standards were frozen and cut to the desired thickness for ablation and construction of multi-point calibration curves.

An Fe-fed mouse model for Parkinson's disease (PD) was used to demonstrate the characteristics of the technique. C57BL6 mice were fed a diet high in Fe during development and were treated with both clioquinol (CQ) and L-DOPA, both of which are thought to chelate Fe in the brain. Results showed a decrease in Fe in the treated animals within the region of the brain called the substantia nigra (SN), which is adversely affected in PD.

A 6-hydroxydopamine (6-OHDA) model for PD was also examined. 6-OHDA is directly injected into the rodent brain, stimulating the loss of cells within the SN. Imaging of sections taken from 6-OHDA lesioned animals showed a significant increase in Fe within the SN bilaterally when compared to control animals.

In summary, elemental bio-imaging is a new method that can be applied to tissue sections from many sources, including humans. The technique has the potential to assist biologists in identifying possible new biomarkers for disease, related specifically to trace elements.